THE TEMPORARY CONCENTRATION OF SEA-SALTS ABOUT *ARBACIA* EGGS.

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I. Introduction.

If the precipitates prepared by Miss Woodward[2] and myself[3] have been correctly understood, Lillie's hypothetical fertilizin[4] is a mixture of at least two chemical entities. On this view, therefore, we cannot assume that the sperm-agglutination test, however reliable for agglutinin, is necessarily also a measure of the concentration of the associated lipolysin. The ratio $\frac{\text{agglutinin}}{\text{lipolysin}}$, may of course be a constant; yet we cannot know this until we find out.

With this problem in mind, I began, last summer, to search for other methods; the ideal being some convenient procedure which would eliminate the physiological variables and leave to the observer nothing except a reading. Experiments on specific gravity, surface tension and viscosity naturally suggested themselves. Changes in one or all of these properties of sea-water might be expected as exudate leaves the eggs and becomes distributed in the solvent. Such changes, plus or minus, should stand, within certain limits, in some direct or inverse relationship with the concentration of the organic constituents of the secretion. A comparison of such values with results gotten by the sperm-agglutination test for the same secretions and for solutions of precipitated agglutinin having the same specific gravity, surface tension, or viscosity, could then be used to throw light on the ratio $\frac{\text{agglutinin}}{\text{lipolysin}}$.

[1] From the Biological Laboratory of Amherst College, Amherst, Mass.

[2] Woodward, A. E., Studies on the Physiological Significance of Certain Precipitates from the Egg-Secretions of *Arbacia* and *Asterias*. J. Exp. Zoöl., Vol. 26, p. 459.

[3] Glaser, O., The Duality of Egg-Secretion. Am. Nat., Vol. LV., p. 368.

[4] Lillie, F. R., The Mechanism of Fertilization. Science, Vol. XXXVIII, p. 524.

II. The Diluent Effect of *Arbacia* Eggs.

I tried practically all the familiar variants of the drop and capillary methods for surface tension; the rates of flow, of falling plungers, and of capillary rise, for viscosity; the Westphal balance, for specific gravity. Yet despite the precautions taken to insure comparable measurements, my results remained inconsistent. Even the specific gravity readings were irregular and their sense totally contrary to expectations. In fact the specific gravity of sea-water in process of receiving exudate from the eggs, rarely rose, never remained constant, and almost invariably fell. The records in Table I. show the discrepancies.

Table I.

No.	Ratio.	Secretion Time.	Temperature.	Specific Gravity.	
				Sea-Water.	Exudate.
A.....	$\frac{\text{eggs}}{\text{sea-water}} = 1/10$	1 hr.	23°	1.0226	1.0222
B.....	" = 1/10	1 hr.	23°	1.0226	1.0217
C.....	" = 1/10	1 hr.	23°	1.0226	1.0222
D.....	" = 1/10	2 hrs.	21.5°	1.0227	1.0231
E..	" = 1/10	30 min.	23°	1.0231	1.0222
E..	" = 1/10	1 hr.	23°	1.0231	1.0221
E..	" = 1/10	1½ hrs.	23.5°	1.0229	1.02195
E..	" = 1/10	2½ hrs.	23.7°	1.02275	1.0224

It is apparent that the introduction of *Arbacia* eggs into sea-water results in a slight decrease of specific gravity and that this decrease may be compensated or even over-compensated with the lapse of time. This is illustrated by series *E*, based on a single set of eggs, and by *D*. If their correctness could be established, these observations would account for the inconsistencies in surface tension and viscosity determinations; yet the fall in specific gravity, the irregularities in the magnitude of the fall, and the compensations would remain to be accounted for.

III. The Chlorine Deficit in Egg-Secretion.

There are four possible explanations. First, the specific gravity determinations may have been wrong. Secondly, the diluent effect of the eggs might be due to a liberation of heat. And finally,

the reductions in density may have resulted from one or the other or both of two causes; either salts are removed from the sea-water directly by the eggs, or the exudate itself affects the sea-water such that the specific gravity must fall.

The first two assumptions were entirely ruled out by subsequent developments. I shall limit myself therefore to a discussion of the other possibilities. Do *Arbacia* eggs abstract salts from the sea-water or is the reduction in specific gravity an effect traceable to the materials which the eggs secrete?

Just how the presence of exudate might reduce specific gravity, is more or less uncertain in detail. Nevertheless this possibility must be reckoned with, both in its thermal, as well as more narrowly chemical, aspects. Salts dissolved in water apparently bring about a "contraction" of the solvent.[5] Where ionization is incomplete, this effect, though marked, is not easily calculable; in dilute solutions, however, the total "contraction" is additively the sum of specific effects or moduli of the individual ions. Thus a gram-molecule of a salt with molecular weight M in m grams of water produces a change of volume Δ_v such that

$$\Delta_v = \frac{M - m}{S} - \frac{m}{S_o},$$

where S is the density of the solution at a given temperature and S_o the density of pure water at the same temperature.

Since undissociated molecules also have a "contractile" effect, and since each ion has a specific modulus, it would be quite possible to bring about a reduction in the specific gravity of sea-water by the addition of some agent that disturbs, selectively or otherwise, the ion-salt equilibrium. Our problem then narrows down to this: is the observed decrease in specific gravity associated with a genuine salt-deficit in the solution or is it the outcome, direct or indirect, of a physical-chemical rearrangement among the free solutes?

If real, and essentially non-selective, a salt-deficit in the solution should be detectable by the titration of the chlorides. For this purpose I used $n/20$ $AgNO_3$; and two or three drops of 10 per cent. K_2CrO_4, in doubly distilled water, as indicator.

[5] Nernst, W., Theoretical Chemistry. MacMillan and Co., London, 1895, p. 331.

Special precautions were taken since the specific gravity readings suggested that differences, if at all discoverable by titration, would be small. For this reason the measurements throughout were made with the same burettes and pipettes and comparable titrations always at the same temperature. Moreover it soon became apparent that the preparation of the eggs could not be carried out by any of the methods in ordinary practise. I therefore washed the sea-urchins first very thoroughly in a stream of running fresh water after which they were completely submerged in dishes for from three to five minutes. The bath was followed by partial drying and the complete removal of the spines by means of a coarse cloth. The naked tests were then carefully wiped with a clean towel and placed in an inverted position in individual Syracuse watch crystals. This procedure may appear cumbersome. However, it consumes very little more time than the usual methods of preparation and is the only way in which eggs absolutely free from detritus, traces of sea-water, dermal and other secretions, can be gotten. Incidentally the method has a further advantage; the brief immersion in fresh water causes the sea-urchins to shed their sexual products in unusual quantity and with the greatest promptness. Indeed one must work quickly in

TABLE II.

Series.	Ratio.	Secretion Time, Minutes.	Chlorine, in c.c. $n/20$ $AgNO_3$, per c.c.		Deficit per c.c. in 1/10 Milligrams of Chlorine.
			Sea-water.	Secretion.	
I.....	$\frac{\text{eggs}}{\text{sea-water}} = 1/7$	15	10.5	10.3	−4
II.....	" = 1/7	15	10.5	10.4	−2
III.....	" = 1/30	60	11.0	10.9	−2
IV.....	" = 1/8	60	10.1	9.8	−5
IV.....	" = 1/8	60	10.0	9.8	−4
IV.....	" = 1/8	60	10.0	9.9	−2
IV.....	" = 1/8	60	10.2	9.8	−7
V.....	" = 1/3	60	10.5	10.1	−7
VI.....	" = 1/8	90	10.5	10.3	−4
VII.....	" = 1/20	120	11.0	10.9	−2
VIII.....	" = 1/8	150	9.9	9.8	−2
IX.....	" = 1/5	150	10.6	10.4	−4
IX.....	" = 1/5	150	10.6	10.5	−2
X.....	" = 1/8	180	10.5	10.4	−2
XI.....	" = 1/2	180	10.6	10.5	−2
XI.....	" = 1/2	180	10.7	10.4	−5

order to prevent the loss of good material through premature shedding of the eggs and sperm.[6]

With these precautions the titration of the chlorides yields perfectly consistent results.

Considering the sources of error and especially the difficulty of measuring the volume of a large number of eggs, the constancy in the sense of these differences is impressive. There is unquestionably a chlorine-deficit in egg-secretion.

IV. The Origin of the Chlorine-Deficit.

How does the chlorine deficiency arise? There is a presumption in favor of attributing it to the eggs; on the other hand, if these eliminate substances capable of masking the chlorine, $AgNO_3$ would give no more clue to its presence than in the titration of chloroform or trichloracetic acid. The problem is soluble by two very simple tests.

If the chlorine is removed by the eggs rather than masked by the exudates, it should be possible to prepare egg-secretions without a chlorine-deficit. To accomplish this the eggs should be exposed to sea-water until all the chlorine which they are able to hold has presumably been taken up. Such eggs if subsequently permitted to secrete into a fresh volume of sea-water should remove no chlorine whatever on their second exposure.

The reasoning is justified by the following experiment in which 1 c.c. of control sea-water, 1 c.c. of first sea-water and 1 c.c. of second, are all expressed in terms of $AgNO_3$ $n/20$.

Table III.

Ratio.	Time.	Control Sea-water.	1st Sea-water.	2d Sea-water.
$\frac{\text{Eggs}}{\text{Sea-water}} = 1/8$	11 A.M.	10.5	10.5	
	11:30	10.5	10.3 Eggs transferred to 2d Sea-water.	
	11:30			10.5
	12:00	10.5		10.5

[6] The efficacy of a three- to five-minute submersal in fresh water was first noticed by my colleague, Miss Sampson.

This result however does not yet solve the problem. It might be argued that the substance which masks the chlorine is secreted only by eggs newly shed from the ovary. If true, the failure to remove chlorine in the above experiment from the second volume of sea-water could be attributed to the absence of the chlorine-masking secretion.

A direct test of this idea is easily made, for if the deficit were, in reality, only apparent, it should be possible by complete evaporation to recover from equal volumes of sea-water and of secretion, equal quantities of sea-salts. This, as the following comparison shows, is not the case.

TABLE IV.

	Chlorine per c.c. as $AgNO_3$ $n/20$.	Total Salt per c.c.
Sea Water	10.6 c.c.	.0387 gram
Secretion	10.4 c.c.	.0370 gram

We must conclude then that the chlorine is not masked by the organic materials in the secretion, but is removed from solution by the eggs. Moreover, if we arbitrarily assume that the eggs remove only KCl, which in relation to its chlorine content is the heaviest of the salts present, there would still remain a discrepancy between the total salt-deficit of 1.7 milligrams per c.c. of secretion and the loss attributable to KCl alone. The total deficit therefore does not appear to result from a selective action on the part of the eggs. On the contrary, we must believe that all the salts are affected in proportion to their concentration in sea-water and their capacity for being removed by the particular egg-mechanism involved in the process.

V. THE MECHANISM BY WHICH SALTS ARE REMOVED FROM THE SEA-WATER.

Although an inspection of Table II. suggests that the chlorine deficit does not increase after the first fifteen minutes of exposure, the actual state of affairs can be rendered much clearer by reducing the values given to a common basis. We will assume an ample supply of chlorine; also, for the sake of comparison, that in one hour, 1 c.c. of eggs can remove from 1 c.c. of sea-water as much chlorine as from twenty; and further, that in 1 hour 1

c.c. of eggs removes one third as much as in three. On the basis of these assumptions and the actual titrations, we can construct a curve showing the comparative amounts of chlorine which, under the conditions imagined, 1 c.c. of eggs would abstract from 1 c.c. of sea-water in one hour, if the rate of removal for that hour were constantly the rate deducible from the determinations actually made after exposures of 15, 60, 90, 120, 150, and 180 minutes.

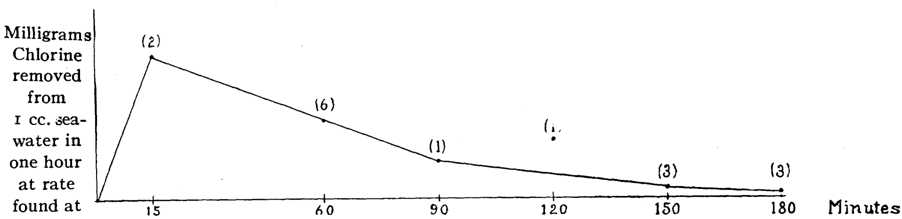

FIG. 1. Curve showing gradual loss in the power of eggs to abstract salts from sea-water.

The number of titrations upon which the points indicated in the curve rest, is given in brackets. Clearly there is a steady approach to zero. Since the rate of chlorine removal by eggs immersed for 180 minutes falls from the height which it had reached after 15 minutes it follows that the process of salt-abstraction is reversible. However, there is no equilibrium. We must suppose therefore that the chlorine-removing mechanism slowly breaks down.

Within the time-limit of these experiments, the only part of the egg-system known to undergo dissolution is the jelly which surrounds each newly shed egg. This chorion disintegrates slowly in sea-water. Fortunately it can also be readily removed by moderate shaking. I therefore compared the chlorine deficits produced by equal volumes of normal and dechorionized eggs in equal volumes of sea-water.

The experiment, of course, involves two errors: in the first place one cannot be certain that the jelly has actually been removed from every egg; and secondly, its removal from a considerable number renders it certain that a given volume of dechorionized ova will contain a larger number of cells than an

equal volume of normal eggs. Both errors really operate against any à priori idea that the jelly is responsible for the removal of the salts. If then the result is clear cut nevertheless, it would appear that the experiment is decisive.

Two such comparisons are given in Table V.

TABLE V.

CHLORINE PER C.C. IN TERMS OF $AgNO_3$ $n/20$.

	Sea-Water Control.	Sea-Water with Normal Eggs.	Sea-Water with Chorion-free Eggs.
After 30 minutes......	11.0	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{14.8\text{ c.c.}} = 10.9$	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{14.8\text{ c.c.}} = 11.0$
After 2 hours.........	11.0	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{10\text{ c.c.}} = 10.9$	$\frac{\text{eggs}}{\text{sea-water}}\ \frac{.2\text{ c.c.}}{10\text{ c.c.}} = 11.0$

A further test seemed desirable. If the jelly takes up the salts, it should be possible to demonstrate their presence in the chorion. The method was that of Macallum[7] in which the reagent, $n/10$ $AgNO_3$ is acidulated, per liter, with 25 c.c. of 60 per cent. HNO_3 in order to avoid any confusion that might result from possible phosphate precipitates or combinations of the silver with proteins or their constituent parts.

The eggs after being carefully drained were gradually transferred to absolute alcohol, and after hardening, subsequently treated with Macallum's reagent for half an hour. After this they were placed on a glass slide, cleared in glycerine under a cover slip, and exposed to direct sunlight for 30 minutes. The distribution of the reduced silver is shown in Fig. 2.

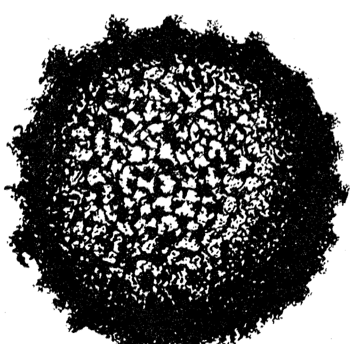

FIG. 2. *Arbacia* egg with chlorides indicated in chorion.

[7] Macallum, A. B., Die Methoden der Biologischen Mikrochemie. Abderhalden's Handb. d. Bio-chem. Arbeitsmethoden, 1912, p. 1100.

Conclusions.

Any attempt to standardize *Arbacia* egg-secretion by physical methods must take into account the fact that these eggs temporarily lower the specific gravity of sea-water. This renders unreliable any immediate application of methods depending on surface tension, viscosity, or specific gravity.

The decrease in density is accompanied by a measureable chlorine deficiency in the solution and by a greater shortage of total salt. We cannot attribute these effects directly or indirectly to the substances which the eggs eliminate since a preliminary exposure of the eggs to sea-water enables us to produce on second exposure a secretion without salt-deficit. Moreover, the results of total evaporation show that the salts were definitely out of the solution and that there is no selective abstraction by the eggs other than that dependent on the proportions in which the several salts are present and their capacity for being removed by the particular egg-mechanism which is involved in the process.

This mechanism is the chorion, for eggs deprived of their jelly by shaking do not cause a salt-deficit. Localization by means of $AgNO_3$ $n/10$ in the presence of HNO_3 demonstrates the concentration of chlorides about the eggs.

The concentration is temporary since the chorion within a few hours normally undergoes disintegration in sea-water. As the result of this the salts are dispersed and the specific gravity of the sea-water may return to normal. A rise above normal may be attributed to the presence of exudate in solution.

In time these facts may find an application in the theory of fertilization. For the present they are presented without theoretical bias although there are unavoidable suggestions in the fact that the concentration of sea-salts immediately about an *Arbacia* egg is temporarily and measurably greater than the concentration of the same salts in the surrounding sea-water.

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